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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Simon S. et al. EXAMINER: Wehbe, Anne Marie Sabrina

SERIAL NO.: 09/982,120 ART UNIT: 1632

Filed: October 17, 2001

For: ANIMALS, CELLS AND METHODS FOR PRODUCTION OF DETECTABLY-LABELED ANTIBODIES

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VIRGINIA 22313-1450

SIR:

I, SANFORD SIMON, hereby declare and state that:

1. I am a professor and Head of the Lab of Cellular Biophysics at Rockefeller University having received my Ph.D. degree from New York University Medical Center in 1984. After that I was a postdoctoral fellow at Rockefeller University. My full curriculum vitae is attached hereto as Exhibit A.

2. My principal area of research is cellular biophysics, and among other positions I serve as reviewer in numerous funding agencies of many countries, including the National Science Foundation, The National Institutes of Health, and DARPA. I also have served as reviewer for numerous scientific journals including Nature, Science, Cell, Journal of Cell Biology, Nature Biotechnology, Journal of Clinical Investigation, Molecular Biology of the Cell, Journal of General Physiology, Proceedings of the National Academy of Sciences (USA) , amongst many others.

3. In the course of my activities, I have been listed as inventor on several patent applications, including the one noted above entitled "ANIMALS, CELLS AND METHODS FOR PRODUCTION OF DETECTABLY-LABELED ANTIBODIES",

having U.S. Serial Number 09/982,120, which claims priority to U.S. provisional application Serial Number 60/241,053, filed on October 17, 2000.

4. I have reviewed the disclosure of the present application, with particular emphasis on the identification of subject matter that could directly be applied to the genetically engineered animals and antibody producing cells obtained from these animals, and more specifically on the importance of these animals for generation of detectably labeled antibodies specific for preselected antigens.

Thus, given the information provided in the present application, it would be possible for one skilled in the art to prepare the animals and antibody producing cells from these animals that may be useful for in vitro and diagnostic testing using the detectably labeled and antigen specific antibodies disclosed in this application. In my opinion, the disclosures of this current application are sufficient to enable one skilled in the art to make or use the invention described and concomitantly provide to the practitioner teachings that could be applied for the indicated purposes.

5. In this regard, and in corroboration of the disclosure of this current application, I have conducted additional experiments in my own laboratory to further confirm the disclosure in this application and which supports the utility of the genetic constructs described herein for in vitro or diagnostic purposes. Particularly, my laboratory has conducted experiments which demonstrate that three proteins can be simultaneously expressed in tandem at the constant region at the end of the kappa light chain. This genetic construct contains the MXE intein, a chitin binding protein and green fluorescent protein. In the plasmid there is a Neo selection marker that is upstream of the kappa light chain. We have the Neo cassette flanked by LoxP sites and after generation of the ES-cells and then expression in mice, we delete Neo cassette with Cre. We have found in our preliminary experiments that Neo upstream affects expression of the Kappa light chain. All constructs have DTA (diphtheria toxin) for a negative selection.

The CBD (chitin binding domain) is used to purify the antibodies. It has proven to be an effective technique for one-step purification of proteins. The GFP is a fluorescent marker that is used both to tag the Ab in subsequent uses, but also to mark the antibodies during the screening assays (this SIGNIFICANTLY accelerates the speed and ease of screening for antibody during the subcloning). The MXE intein is part of a split

intein (a self-splicing protein) that can be used to covalently bind the kappa MXE to the other split part of the MXE intein. For example if we conjugate another fluorophore (such as an organic fluorophore or a quantum dot) to the other part of the MXE intein, it will be covalently linked to it. The reaction is very specific. We setup the construct in this way so that the chitin binding domain and the GFP will then be cleaved off: The cleavage of the GFP is used to assay the kinetics and completion of the reaction.

We have also made another construct for which the GFP is between the Kappa and the MXE intein. This construct will keep the GFP attached. It will be used for when we want to use the intein to introduce a toxin, radiolabel or another probe onto the antibody.

6. The results shown herein provide further proof for the generation of an additional chimeric antibody that contains at least one detectable label ie. GFP. Furthermore, the generation of this construct provides support for the production of a chimeric antibody that contains both GFP as well as an MXE intein and a chitin binding domain. The original application refers to this type of construct on page 15, lines 12-15. These results fully support our earlier data provided in the present application. Furthermore, the data presented herein, as well as the data provided in the present application, fully support the enablement and use of chimeric antibodies containing at least one detectable label, and further support a chimeric antibody containing a detectable label (GFP) as well as an intein and a chitin binding domain.

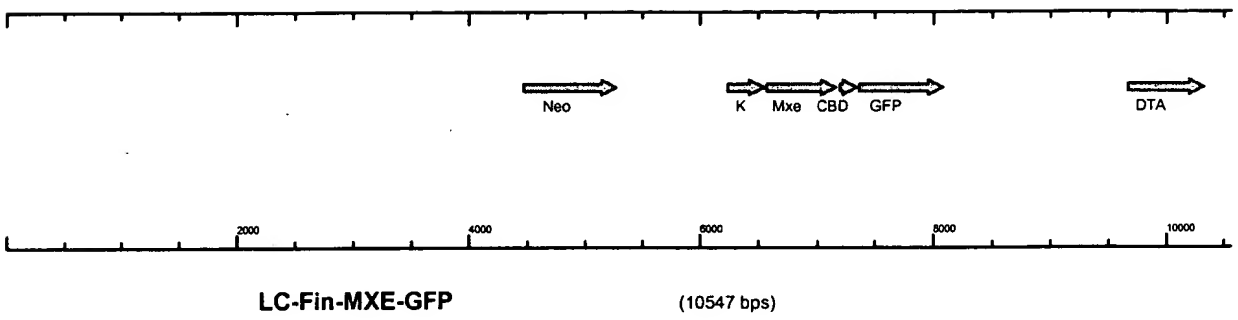
The advantages of this technology are as follows. Such a genetic construct provides for the ability of this type of chimeric antibody to be useful for not only detection of the specific antigen in vitro in a diagnostic setting, but also is useful for antibody purification due to the presence of the chitin binding domain or for introduction of a toxin or radiolabel or other probe onto the antibody due to the presence of the intein.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18 of

the U.S. Code, Section 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: ___April 13th, 2004_____

Sanford Simon



This is a construct we have used in a second generation of mice. In short, the goal was to have three proteins simultaneously expressed in tandem at the constant region at the end of the kappa light chain: The MXE intein, a chitin binding domain and GFP.

In the plasmid there is a Neo selection marker that is upstream of the kappa light chain. We have the Neo cassette flanked by LoxP sites and after generation of the ES-cells and then expression in mice, we delete Neo cassette with Cre. We have found in our preliminary experiments that Neo upstream affects expression of the Kappa light chain. All constructs have DTA (diphtheria toxin) for a negative selection.

The CBD (chitin binding domain) is used to purify the antibodies. It has proven to be an effective technique for one-step purification of proteins. The GFP is a fluorescent marker that is used both to tag the Ab in subsequent uses, but also to mark the antibodies during the screening assays (this SIGNIFICANTLY accelerates the speed and ease of screening for Ab during the subcloning). The MXE intein is part of a split intein (a self-splicing protein) that can be used to covalently bind the kappa MXE to the other split part of the MXE intein. For example if we conjugate another fluorophore (such as an organic fluorophore or a quantum dot) to the other part of the MXE intein, it will be covalently linked to it. The reaction is very specific. We setup the construct in this way so that the chitin binding domain and the GFP will then be cleaved off: The cleavage of the GFP is used to assay the kinetics and completion of the reaction.

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EXHIBIT A

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Sanford M. Simon	Professor, Head of Laboratory

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Princeton University	BA	1977	Neuroscience
NYU Medical Center	MA	1980	Physiology & Biophysics
NYU Medical Center	Ph. D.	1984	Physiology & Biophysics
Rockefeller University	Postdoc	1984-1989	Cell Biology/Gunter Blobel

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

2001 – present. Professor, The Rockefeller University.
1994 – 2000. Associate Professor, The Rockefeller University Walter Annenberg Research Professor
1989 – 1994. Assistant Professor, The Rockefeller University
1984 – 1989. Post-doctoral associate, The Rockefeller University with Gunter Blobel.
1977 – 1984. Graduate Student, Dept of Physiology & Biophysics, NYU Medical Center with Rodolfo Llinas.
1972 – 1977. Undergraduate, Princeton University Research with Alan Gelperin & Barry Jacobs.

B. Selected peer-reviewed publications (in chronological order). (abbreviated list).

M. Fix, T. J. Melia, J. K. Jaiswal, J. Z. Rappoport, D. You, T. Söllner, J. E. Rothman, and S. M. Simon. Imaging of single membrane fusion events mediated by SNARE proteins. Proc.Natl.Acad.Sci.U.S.A. 2004.(In Press)

JZ Rappoport, SM Simon, A. Benmerah. Understanding living clathrin-coated pits. Traffic 2004.(In Press)

M. S. Wollenberg and S. M. Simon. Signal sequence cleavage of peptidyl-tRNA prior to release from the ribosome and translocon. J.Biol.Chem. 2004.(In Press)

H. Mattoussi, I. L. Medintz, A. R. Clapp, E. R. Goldman, J. K. Jaiswal, S. M. Simon, and J. M. Mauro. Luminescent Quantum Dot-Bioconjugates in Immunoassays, FRET, Biosensing and Imaging Applications. Journal of the Association for Laboratory Automation 9:28-32, 2004.

J. K. Jaiswal, H. Mattoussi, J. M. Mauro, and S. M. Simon (2003). Long-term multiple color imaging of live cells using quantum dot bioconjugates. Nat.Biotechnol. 21 (1):47-51.

J. Z. Rappoport and S. M. Simon. (2003) Real-time analysis of clathrin mediated endocytosis during cell migration. J.Cell Sci. 116:847-855.

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G. Kreitzer, J Schmoranzer, SH Low, X Li, Y Gan, T Weimbs, SM Simon, and E. Rodriguez-Boulan. Three-dimensional analysis of post-Golgi carrier exocytosis in epithelial cells. Nat.Cell Biol. 5 (2):126-136, 2003.

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A. Rajagopal and S. M. Simon. Subcellular localization and activity of multidrug resistance proteins. Mol.Biol.Cell 14 (8):3389-3399, 2003.

J. K. Jaiswal, N. W. Andrews, and S. M. Simon. (2002) Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. J.Cell Biol. 159 (4):625-635.

E. M. Kanner, I. K. Klein, M. Friedlander, and S. M. Simon (2002). The amino terminus of opsin translocates "Posttranslationally" as efficiently as cotranslationally. Biochemistry 41 (24):7707-7715.

N. F. De Souza and S. M. Simon (2002) . Glycosylation Affects the Rate of Traffic of the Shaker Potassium Channel through the Secretory Pathway. Biochemistry 41 (38):11351-11361.
A. Rajagopal, A. C. Pant, S. M. Simon, and Y. Chen. (2002) In Vivo Analysis of Human Multidrug Resistance Protein 1 (MRP1) Activity Using Transient Expression of Fluorescently Tagged MRP1. Cancer Res. 62 (2):391-396.
Y. Chen, A. C. Pant, and S. M. Simon. (2001) P-glycoprotein does not reduce substrate concentration from the extracellular leaflet of the plasma membrane in living cells. Cancer Res. 61 (21):7763-7769.
M. A. Lampson, J. Schmoranzer, A. Zeigerer, S. M. Simon, and T. E. McGraw (2001) . Insulin-regulated Release from the Endosomal Recycling Compartment Is Regulated by Budding of Specialized Vesicles. Mol.Biol.Cell 12 (11):3489-3501.
D. K. Marciano, M. Russel, and S. M. Simon (2001) . Assembling filamentous phage occlude pIV channels. Proc.Natl.Acad.Sci.U.S.A. 98 (16):9359-9364.
Goulian, M. and S. M. Simon (2000). Tracking single proteins within cells. Biophys.J. 79:2188-2198.
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